



Light and ultrastructural analysis of *Myxobolus insignis* (Myxozoa), infecting the Amazonian Fish *Semaprochilodus insignis* (Prochilodontidae)

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Abstract

A myxosporean infecting the gill filaments of the freshwater teleost *Semaprochilodus insignis* collected in the Trombetas River (Central Amazonian Region, Brazil) is described using light and electron microscopy. The spores were ovoid in frontal view with round extremities and measured $15.4 \pm 0.6 \mu\text{m}$ in total length, $12.4 \pm 0.5 \mu\text{m}$ wide and $8.1 \pm 0.7 \mu\text{m}$ thick; the spore valves (up to $0.4 \mu\text{m}$) were surrounded by a uniform dense layer with variable thickness up to $\sim 1.0 \mu\text{m}$ due to the presence of a complex network of anastomosed microfibrils closely adherent to the valves. Two symmetric polar capsules measured $5.9 \pm 0.4 \mu\text{m}$ long and $3.4 \pm 0.5 \mu\text{m}$ wide, each having a polar filament with 7-8 coils slightly obliquely to the longitudinal axis of the polar capsule. The polar capsule wall measured $\sim 0.4 \mu\text{m}$ thick and was constituted by a hyaline substance ($\sim 0.25 \mu\text{m}$ thick) surrounded by a layer of electron dense granular material ($\sim 0.15 \mu\text{m}$ thick). In this paper we present, by the first time, ultrastructural aspects of the spores of *Myxobolus insignis* found in a teleost collected from the Amazonian region, which was previously described based on light microscopy (Eiras *et al.* 2005b).

Key words: Trombetas River, Amazonia, gills, myxosporean, *Myxobolus insignis*, spores, parasite

Introduction

Numerous descriptions of myxosporean species have been reported in fish from different geographic areas (Lom & Dyková 2006). Among the myxosporeans, the genus *Myxobolus* is one the most common myxosporean pathogens infecting fishes, possessing a world-wide distribution (Gioia & Cordeiro 1996; Eiras *et al.* 2005a; Lom & Dyková 2006).

The great majority of the different species of *Myxobolus* parasitizing Brazilian host freshwater fishes were described using light microscopy and diagrammatic drawings of their spores to determine the species (Kent & Hoffman 1984; Molnár & Békési 1993; Molnár *et al.* 1998; Cellere *et al.* 2002; Eiras *et al.* 2005b, 2007; Martins & Onaka 2006). Some ultrastructural data of the developmental stages of the different *Myxobolus* spp. have also been provided (Casal *et al.* 1996, 2002, 2006; Azevedo *et al.* 2002, 2009, 2010, 2011; Tajdari *et al.* 2005; Adriano *et al.* 2006, 2009a, 2009b, 2010).

This paper presents ultrastructural data of *Myxobolus insignis* parasite of *Semaprochilodus insignis* collected from the Amazonian region, which was previously described based on light microscopy (Eiras *et al.* 2005b).

Material and methods

Sample collection. During a parasitological survey expedition in the Amazon River during January 2011, numerous fishes of different species were collected. Among them, 15 specimens of *Semaprochilodus insignis* Jardine, 1841 (Teleostei, Prochilodontidae) (15–21 cm long) (Brazilian common name “Jaraqui”) were present. More precisely, the fishes were collected from the Trombetas River (Tributary of the central region of the Amazon River) (01° 45'S/ 55° 53'W), near the city of Oriximiná (State of Pará), Brazil, located about 550 km downstream from the city of Manaus and about 770 km upstream from the Amazon River mouth, Brazil. They were transported live to the laboratory of the Campus of the Federal Fluminense University located in the city of Oriximiná. The fish that died during the trip were placed on ice (2–3 h) before dissection.

Microscopic analysis. Plasmodia and spores found in gills were examined in fresh mounts with a light microscope equipped with Nomarski differential interference-contrast optics (LM-DIC). For transmission electron microscopy (TEM), small parasitized fragments of infected tissues and free spores were fixed in 5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) at 4 °C for 20–24 h, washed overnight with the same buffer, and post-fixed in 2% OsO₄ buffered with 0.2 M sodium cacodylate for 4 h at the same temperature. The infected fragments were dehydrated in an ascending ethanol and propylene oxide series and embedded in Epon. Semithin sections were stained with methylene blue–Azur II and observed under LM-DIC optics. Ultrathin sections, cut with a diamond knife, were stained with both aqueous uranyl acetate and lead citrate and observed in a JEOL 100CXII TEM, operated at 60 kV.

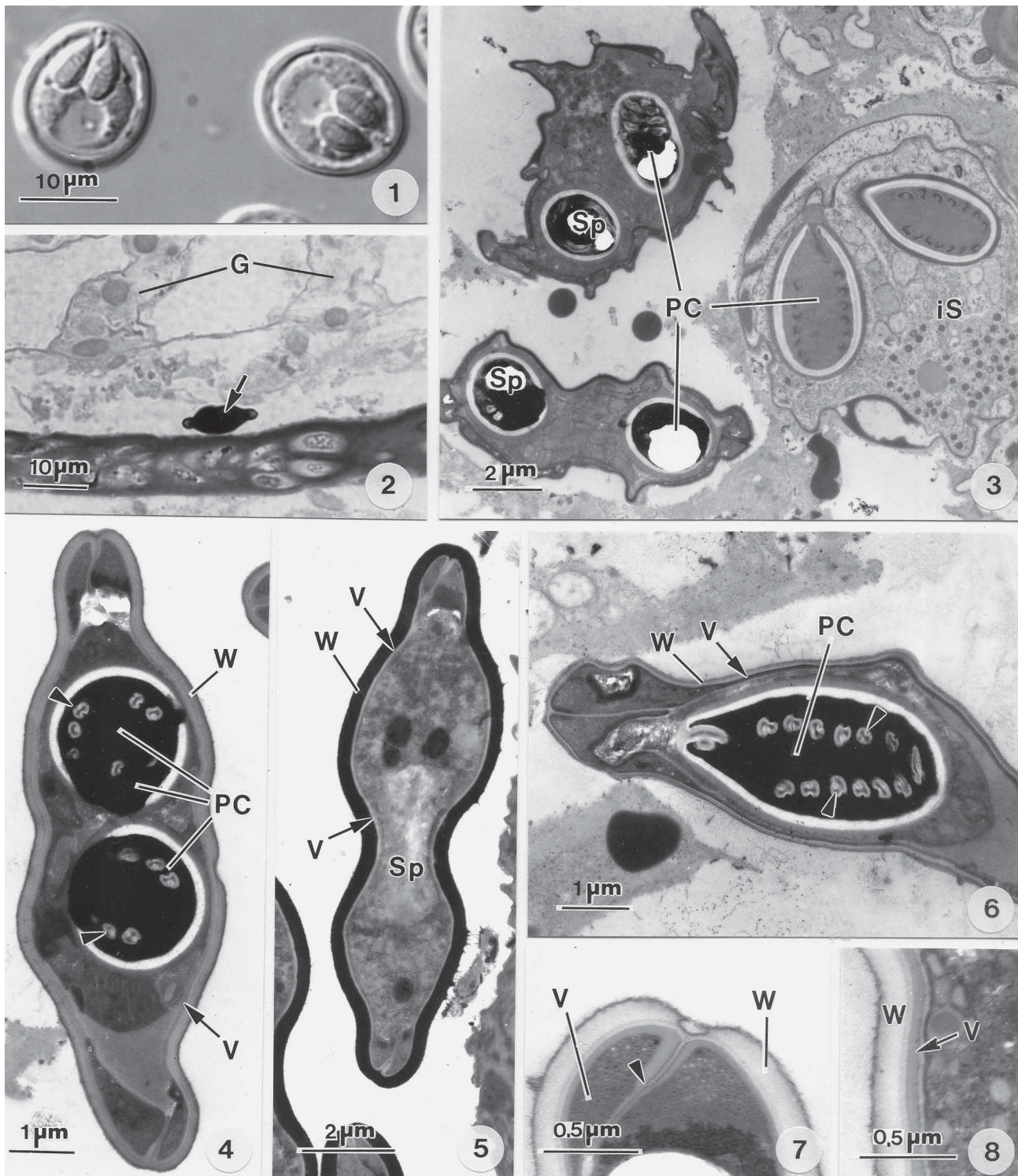
Results

Material studied. The infected fish contained several plasmodia appearing as whitish cysts located in the base of the gill filaments. The plasmodia were at different developmental stages, including immature and mature spores. The specimens of *Semaprochilodus insignis* were observed macroscopically in order to detect the possible presence of parasitic infection. LM-DIC observations demonstrated that 7 of 15 (46.6 %) of fish specimens (4/7 males and 3/8 females) were infected by several myxozoan plasmodia containing spores, which were identified microscopically as *Myxobolus insignis* (Fig. 1).

Light and Ultrastructural data of the spore (Figs. 1–9). Several disporic coelozoic plasmodia infecting the gill filaments contained numerous developmental stages, including spores. The immature and mature spores were observed within plasmodia located on the base of the gill lamellae. The central regions of these plasmodia were mainly occupied by numerous mature spores. Aspects of ruptured plasmodia were observed, but some isolated spores in contact with the gill epithelium were observed (Fig. 2).

Morphological data. The spores were ovoid and measured $15.4 \pm 0.6 \mu\text{m}$ ($n = 50$) in length, $12.4 \pm 0.5 \mu\text{m}$ ($n = 50$) in width and $8.1 \pm 0.7 \mu\text{m}$ ($n = 15$) in thickness (Fig. 2). The mature spores were composed of two unequal shell valves adhering together along the straight suture lines where the asymmetry was easily observed (Figs. 4, 5, 7). In the mature spores, the valves were composed of a thin electron-lucent substance (up to $\sim 0.4 \mu\text{m}$ thick) and surrounded by a layer with variable thickness (up to $\sim 1.0 \mu\text{m}$ thick), formed by a complex electron-dense network of anastomosed microfibrils, closely adherent to the valves (Figs. 3–5). The suture line formed a thick strand that surrounded the central part of the spore (Figs. 4, 5, 7). Two symmetric elongated and equal-sized polar capsules measuring $5.9 \pm 0.4 \mu\text{m}$ long ($n = 15$) and $3.4 \pm 0.5 \mu\text{m}$ wide ($n = 15$), contained a polar filament with 7–8 coils (Figs. 3, 4, 6), oblique to the axis of the polar capsule (PC). The PC presented a circular transverse section and the polar filament had irregular transverse sections (Figs. 4, 6). Several sporoplasmosomes with circular sections ($\sim 50 \text{ nm}$ thick) were distributed randomly in the cytoplasm of the sporoplasm. A schematic drawing of the spore morphology (Fig. 9) was made on observations from light and serial ultrathin sections.

Histopathology. The capillaries located near the cysts appeared compressed showing in some sections a reduced lumen where the blood seemed to be accumulated.



FIGURES. 1–8. Light and transmission electron micrographs of the myxosporean, *Myxobolus insignis*, from the fish *Semaprochilodus insignis* from Amazonian fauna. 1. Free mature spores observed in valvar view by LM-DIC optic. 2. Semi-thin section of free mature spore observed within the epithelial tissues of the gill (G). 3. Ultra-thin section of the internal region of a plasmodium showing one immature spore (iS) and two mature spores and correspondent polar capsule sections (PC). 4. Spore transversally sectioned at polar capsules (PC) level. 5. Spore transversally sectioned at sporoplasm (Sp) level (5) showing there typical structures, as the spore wall (W), shell valves (V) and suture lines (arrowheads). 6. Longitudinal ultrathin section of a polar capsule (PC), showing a dense matrix (*) surrounding the different transverse sections of the polar filament (arrowheads) and the complex apical region of the PC (arrows). Externally the wall (W) and the valves (V) are observed. 7. Ultrastructural detail of a lateral region of the spore showing the suture line (arrowhead), the valves (V) and the wall (W). 8. Ultrastructural detail of a transverse section of the shell wall (W), the valve (V) and the plasmalemma (double arrowhead) of the sporoplasm.

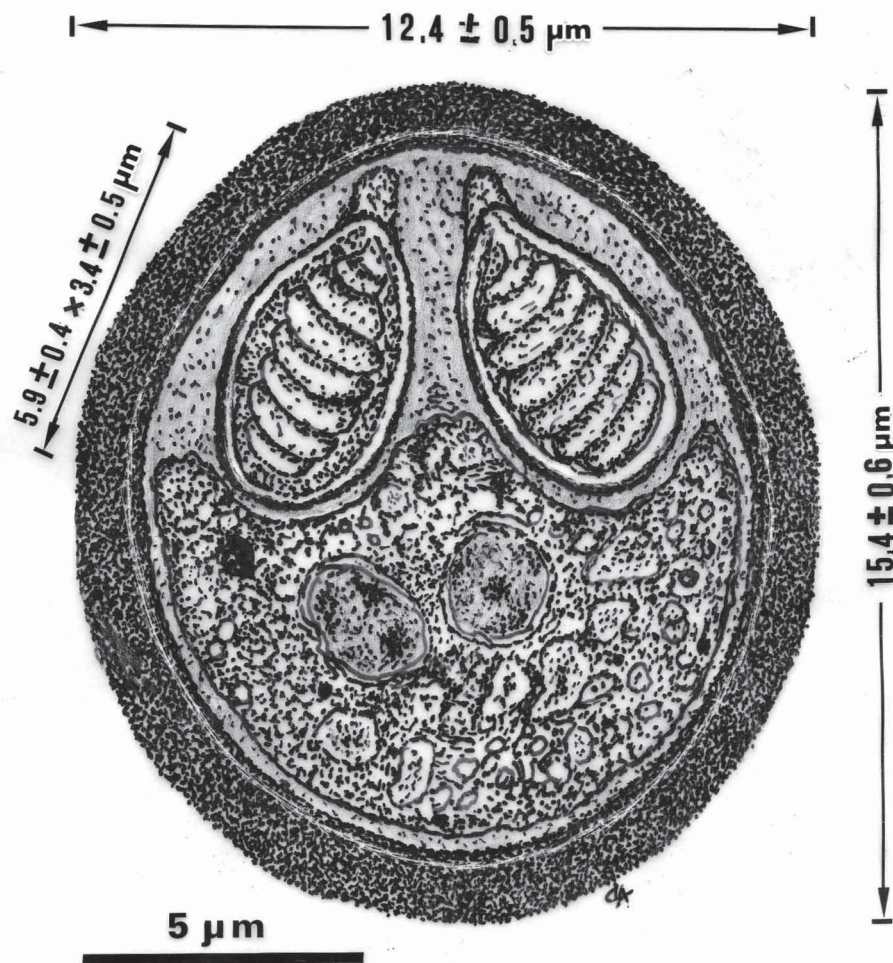


FIGURE 9. Semi-schematic drawing of a valvar view of the spore of *Myxobolus insignis*, a parasite of the freshwater fish *Semaprochilodus insignis* from Amazonian fauna showing the morphologic characters, such as the spore shape and size, two equal polar capsules with six oblique polar filament coils, and the binucleated sporoplasm with several sporoplasmosomes. The spore wall is surrounded by a thick layer of electron-dense anastomosed fine microfibrils.

Discussion

Considering that the myxozoan species described in the present paper was collected from the gill of the Amazonian fish *Semaprochilodus insignis*, from the same hydrographic network and taking in account some morphological aspects of the spores, we believe that our isolate is the same parasite that was previously described as *Myxobolus insignis* (Eiras *et al.* 2005b).

Despite the normal difficulties in comparing data obtained by light microscopy (LM) with data obtained from TEM analyses, the ultrastructural aspects described in this study revealed a slightly different morphology than the one previously described in light micrographs. The spores of the previously described *Myxobolus insignis* and the PC are similar to those of our parasite.

However, two major differences were found between the two descriptions of the spores, one concerning the symmetry or asymmetry of the valves and the other, the thickness of the spore wall. The aspects of the several ultrastructural sections in this study, demonstrated that the valves were asymmetric and not symmetric, as it was reported by LM observation (Eiras *et al.* 2005b). Concerning the thickness of the wall, TEM observations showed that the valves are thin structures ($\sim 0.4 \mu\text{m}$ thick) surrounded by a thick ($\sim 1.0 \mu\text{m}$) layer of fine and anastomosed microfibrils. In LM observations the valvar wall was described as having $1.5\text{--}2 \mu\text{m}$ thick, but the wall organization was confuse with dense layer that surrounded the entire spore and latter disappeared during the spore maturation process, as it was observed in the analyses TEM reported in this study.

The number of polar filament coils was also different when observed by TEM. While the LM description reported 6 coils, TEM allowed the observation of 7-8 coils, a little difference without taxonomic relevance. This study has provided, by the first time, novel information about the ultrastructure of the spore of this species, adding some interesting ultrastructural details not observed in LM.

In conclusion, considering the morphological similarities, spore measurements, polar capsule sizes and shapes and the occurrence in the same organ and location, as well as in the same host species collected in the same hydrographic region (Table 1), we believe, beside some few differences, that these arguments are sufficient to confirm that this parasite belongs to *Myxobolus insignis*, as previously described by Eiras *et al.* (2005b) and that our studies contribute to a more precise description of overall morphology.

TABLE 1. Comparative measurements (in μm) of the spores from *Myxobolus insignis* infecting gills of the fish *Semaprochilodus insignis* collected in two distinct Amazonian regions distant from ~550 km.

<i>Myxobolus insignis</i>	SpL	SpW	SpTh	SpWTh	PCL	PCW	PFc
Eiras <i>et al.</i> 2005b	14 – 15	11 – 12	7 – 8	—	7 – 8	3 – 5	6
Present study	15.2 \pm 0.6	12.4 \pm 0.5	8.1 \pm 0.7	3.5 \pm 0.4	7.8 \pm 0.3	4.0 \pm 0.6	7 – 8

SpL, spore length; SpW, spore width; SpTh, spore thickness; SpWTh, spore wall thickness; PCL, polar capsule length; PCW, polar capsule width; PFc, polar filament coils; —, without data.

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